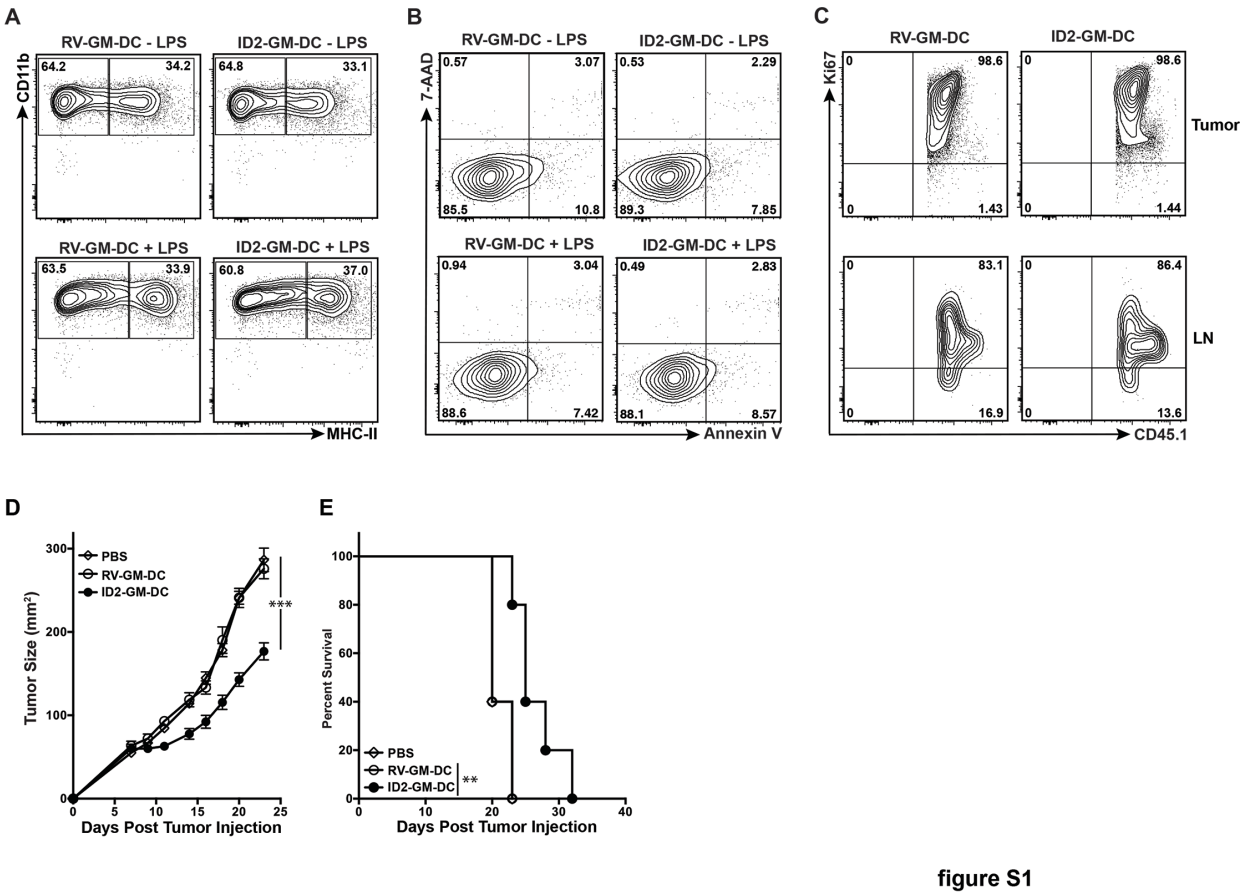

figure S1

Figure S1. Characterization and function of RV-GM-DCs and ID2-GM-DCs. (**A**) Purified RV-GM-DCs and ID2-GM-DCs were stimulated with or without 0.1 μg/ml LPS for 24 hours, and analyzed for CD11b and MHC class II cell surface amounts by flow cytometry. $CD11b^+$ MHC II^+ cells were further gated into $CD11b^+$ MHC II^{lo} and $CD11b^+$ MHC II^{hi} subsets, as indicated. (**B)** Purified RV-GM-DCs and ID2-GM-DCs were maintained without GM-CSF for 24 hours in the presence or absence of 0.1 μg/ml LPS, as indicated. Cell survival was measured by staining with Annexin V and 7-AAD. (**C**) LPS-stimulated RV-GM-DCs and ID2-GM-DCs ($CD45.1^+$) were delivered into melanoma tumors (i.t.) in C57BL/6 ($CD45.2^+$) mice, 7 days after tumor establishment. Twenty-four hours later, $CD45.1^+$ donor cells in the tumor and tumor-draining lymph nodes were analyzed by Ki67 intracellular staining. (**D and E**) C57BL/6 mice were injected s.c. with $1x10^6$ MC38 colon cancer cells, 7 days later RV-GM-DC or ID2-GM-DC vaccines ($2x10^6$ cells per mouse) were delivered i.t. Tumor growth (D) and survival (E) were monitored as indicated in Fig. 1. The survival curves for PBS and RV-GM-DC groups overlap exactly, hence the PBS group is not visible in the figure. Data are representative of 3 independent experiments (A to C), or are means ± SEM of 2 independent experiments with n=10 mice total (5 mice/experiment) (D and E). ** $p< 0.01$, *** $p<0.001$ determined by ANOVA followed by Bonferroni's post test (D) or Kaplan Meier Log-rank test (E).

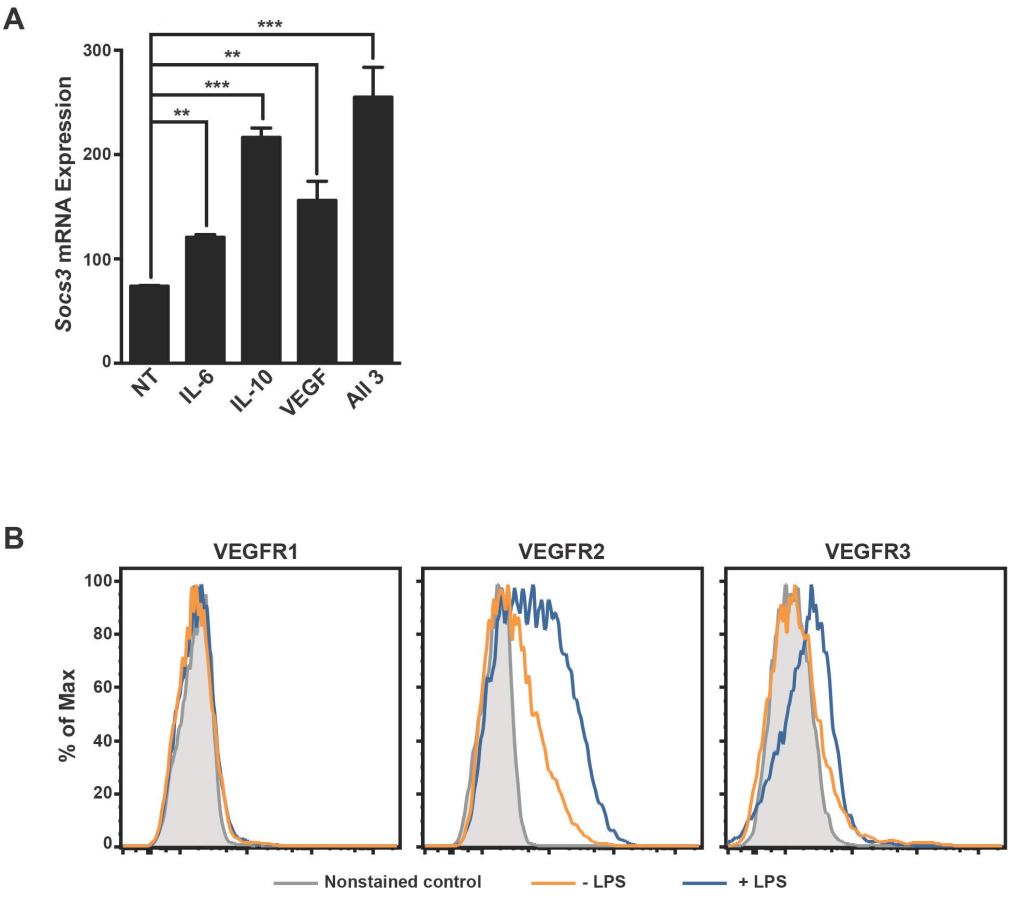

Figure S2. Characterization of STAT3 signaling pathways. (A) GM-CSF-differentiated bone marrow cells (after 7 days culture) were stimulated with IL-6, IL-10, VEGF, or all 3 cytokines for 2 hours, as indicated; expression of the STAT3-responsive gene *Socs3* was evaluated by qPCR. **(B)** GM-CSF-differentiated bone marrow cells were stimulated with or without 0.1 μg/ml LPS for 24 hours. Cell surface amounts of VEGF receptors 1, 2, or 3 within the $CD11c^{+}$ population (GM-DCs) were analyzed using flow cytometry, as indicated. Data are means ± SEM from (A) or are representative of (B) 3 independent experiments. ** $p< 0.01$, *** $p<0.001$, comparing cytokine-treated to untreated (NT) cells (A) by Student's *t*-tests.

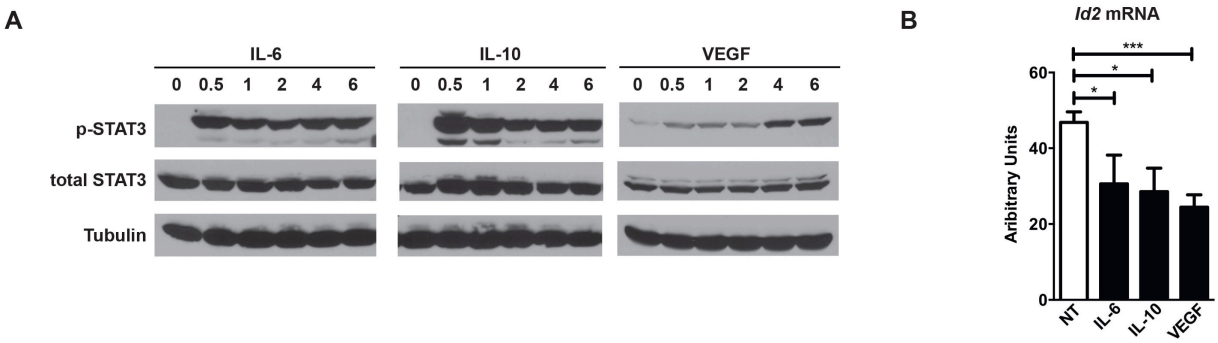

Figure S3. Regulation of *Id2* by STAT3-activating cytokines in D2SC/1 cells. (**A**) D2SC/1 cells were stimulated with IL-6, IL-10 or VEGF (10 ng/ml) for up to 6 hours, as indicated. STAT3 activation was examined by immunoblotting as described in Fig. 2. (**B**) D2SC/1 cells were stimulated with IL-6, IL-10 or VEGF for 6 hours or left untreated (NT), as shown; *Id2* mRNA expression was analyzed by qPCR. Data are representative of (A) or are means ± SEM from (B) 3 independent experiments. * $p< 0.05$, *** $p<0.001$, comparing cytokine-treated to untreated (NT) cells by Student's *t*-tests.

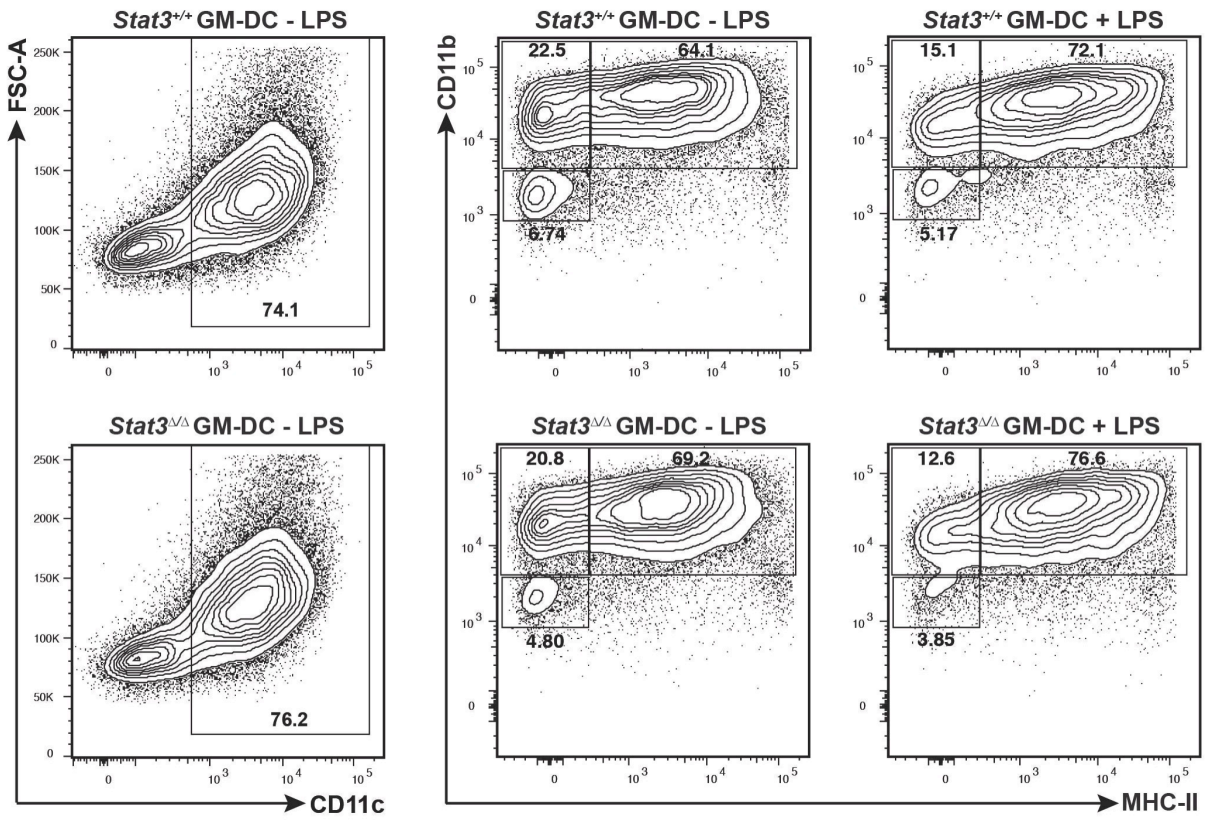

Figure S4. Phenotypic characterization of *Stat3*-sufficient and *Stat3*-deficient GM-DCs. GM-CSF-differentiated bone marrow cells from CD11c Cre^{-} *Stat3*$^{f/f}$ (*Stat3*$^{+/+}$) or CD11c Cre^{+} *Stat3*$^{f/f}$ mice (*Stat3*$^{\Delta/\Delta}$) were cultured with or without 0.1 μg/ml LPS for 4 hours as indicated. The CD11c^{+} fraction (left panel) was further analyzed for CD11b and MHC class II (center and right panels) by flow cytometry, as shown. Data represent 3 independent experiments.

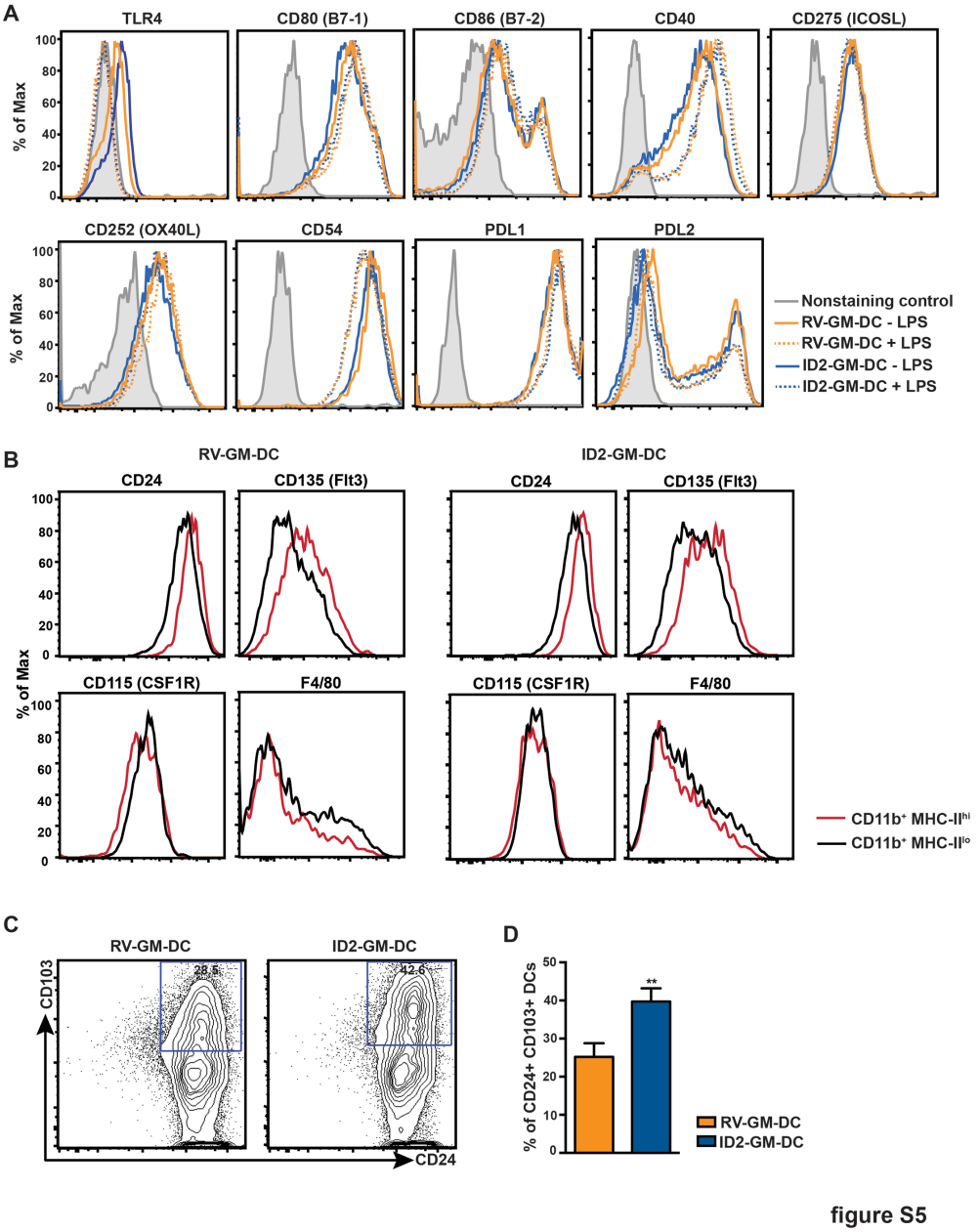

Figure S5. RV-GM-DC and ID2-GM-DC cell surface phenotypes. Purified RV-GM-DCs and ID2-GM-DCs were stimulated with or without 0.1 μg/ml LPS for 24 hours. (**A**) Cell surface amounts of TLR4, costimulatory or coinhibitory molecules were assessed by flow cytometry, as indicated. (**B**) The $CD11b^+$ MHC II^{lo} and $CD11b^+$ MHC II^{hi} populations of purified RV-GM-DCs and ID2-GM-DCs were analyzed for cell surface CD24, CD135, CD115 or F4/80 following LPS stimulation, as indicated. (**C and D)** The proportion of $CD103^+$ $CD24^+$ cells within the $CD11c^+$ (pre-gated) population of RV-GM-DCs and ID2-GM-DCs was analyzed by flow cytometry. Representative results (A to C) and mean MFI ± SEM (D) from 4 independent experiments are shown. (D) ** $p< 0.01$, comparing RV-GM-DC and ID2-GM-DC results by Student's *t*-test.

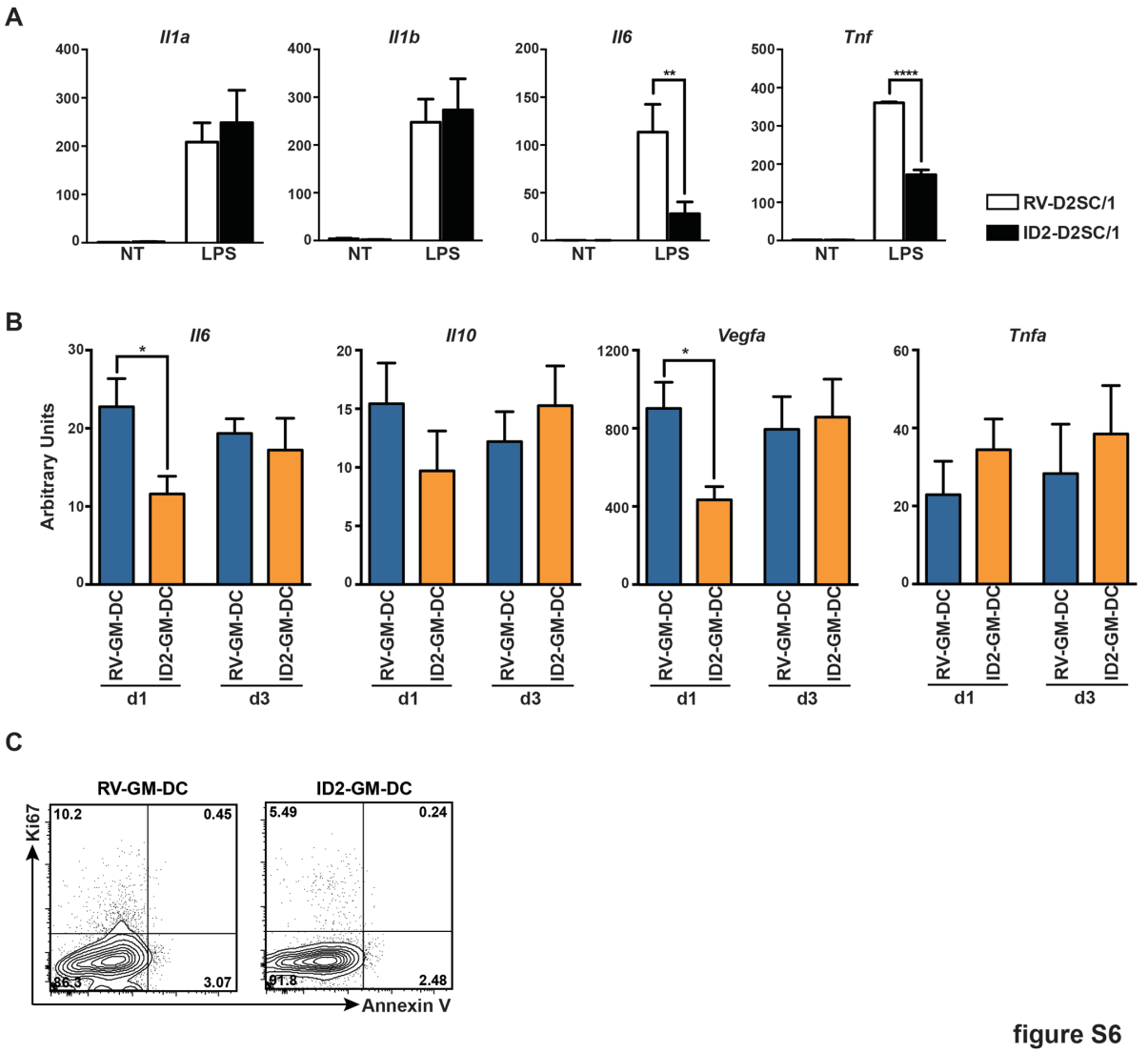

Figure S6. Effects of ID2 in D2SC/1 cells and cytokine production in B16 melanoma tumors. (**A**) D2SC/1 cells engineered to constitutively express ID2 by retroviral transduction (ID2-D2SC/1) or vector-infected controls (RV-D2SC/1) were stimulated with 0.1 μg/ml LPS for 6 hours; cytokine mRNA expression was analyzed by qPCR as indicated. (**B**) B16 melanoma-bearing C57BL/6 mice were immunized with RV-GM-DCs or ID2-GM-DCs as described in Fig. 1C. Tumors were collected at 1 and 3 days following DC vaccination, and *Il6*, *Il10*, *Vegfa*, and *Tnfa* mRNA amounts in total tumor extracts were analyzed by qPCR as indicated. (A and B) Data are means ± SEM from 3 independent experiments. * $p< 0.05$, ** $p< 0.01$, **** $p<0.0001$ for the indicated comparisons, determined by Student's *t*-test. (**C**) $CD45.2^+$ B16-melanoma-bearing mice were immunized with LPS-stimulated RV-GM-DCs or ID2-GM-DCs ($CD45.1^+$) as described in fig. S1C. Twenty-four hours later, $CD45.2^+$ tumor cells were analyzed by Ki67 and Annexin V using flow cytometry. Data are representative of 3 independent experiments.

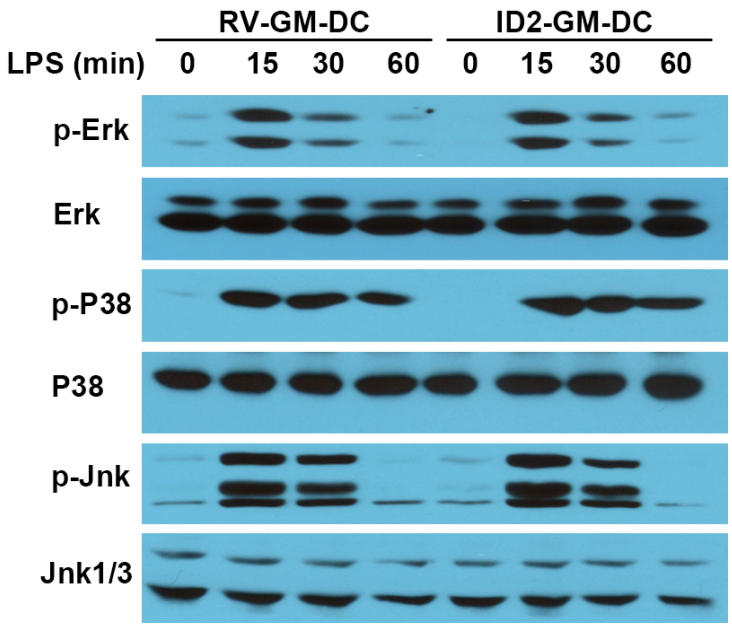

figure S7

Figure S7. MAPK activation in RV-GM-DCs and ID2-GM-DCs. RV-GM-DCs and ID2-GM-DCs were stimulated with LPS (0.1 μg/ml for up to 60 min.) and the activation of ERK, p38 and JNK was examined by immunoblotting. Representative results from 2 independent experiments are shown. p, phosphorylation.

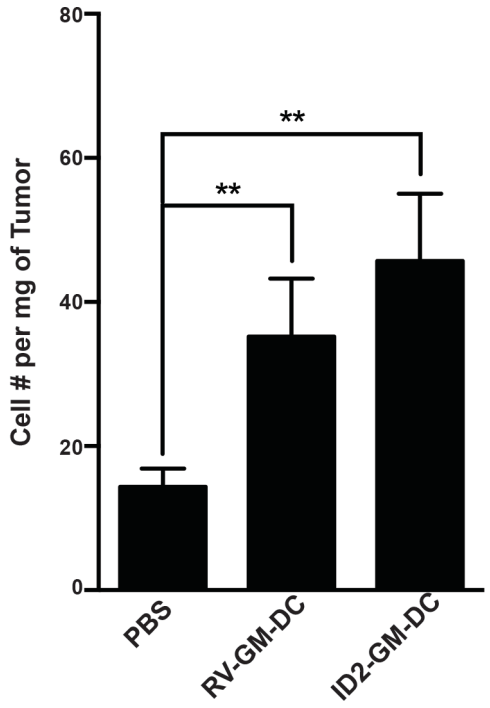

figure S8

Figure S8. Mononuclear cell infiltration in B16 melanomas upon GM-DC vaccination. B16 melanoma-bearing C57BL/6 mice were vaccinated with purified RV-GM-DCs, ID2-GM-DCs or PBS, as described in Fig. 1. Six days after DC vaccine, tumor-infiltrating lymphocyte (TIL) profiles were analyzed by flow cytometry as described in Fig. 5A. Data are means ± SEM from 3 independent experiments, n= 5 mice per experiment. ** $p< 0.01$ for the indicated comparisons, determined by a Student's *t*-test.

Table S1. Oligonucleotide sequences. Sequences of the oligonucleotides used in this study for real-time PCR, ChIP, and EMSAs are listed.

Real-time PCR primers	
Id2 sense	5'-AAAACAGCCTGTCGGACCAC-3'
Id2 antisense	5'-CTGGGCACCAGTTCCTTGAG-3'
Il1a sense	5'-CCAGAAGAAAATGAGGTCGG-3'
Il1a antisense	5'-AGCGCTCAAGGAGAAGACC-3'
Il1b sense	5'-GGTCAAAGGTTTGGAAGCAG-3'
Il1b antisense	5'-TGTGAAATGCCACCTTTTGA-3'
Il6 sense	5'-TGAACAACGATGATGCACTTGC-3'
Il6 antisense	5'-GCTATGGTACTCCAGAAGACC-3'
Il10 sense	5'-TGTCAAATTCATTCATGGCCT-3'
Il10 antisense	5'-ATCGATTTCTCCCCTGTGAA-3'
Il12a sense	5'-ACTAGAGAGACTTCTTCCACAACAAGAG-3'
Il12a antisense	5'-GCACAGGGTCATCATCAAAGAC-3'
Il12b sense	5'-GGAGACACCAGCAAAACGAT-3'
Il12b antisense	5'-TCCAGATTCAGACTCCAGGG-3'
Tnf sense	5'-AGGGTCTGGGCCATAGAACT-3'
Tnf antisense	5'-CCACCACGCTCTTCTGTCTAC-3'
ChIP primers	
Id2 sense	5'-TGTGCAAACCCCACTAATGA-3'
Id2 antisense	5'-CGCTTTTGGGAAGTCACATT-3'
EMAS oligonucleotides	
NF-κB sense	5'-CAACGGCAGGGGAATTCCCCTCTCCTT-3'
NF-κB antisense	5'-AAGGAGAGGGGAATTCCCCTGCCGTTG-3'
NF-Y sense	5'-AAGAGATTAACCAATCACGTACGGTC-3'
NF-Y antisense	5'-GACCGTACGTGATTGGTTAATCTCTT-3'